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Zur Kasse
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Notice of opposition to a European patent

I. Patent opposed

Patent No.	EP 1 392 849
Application No.	02718994.3
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	2 January 2008
Title of the invention	Yeast screens for agents affecting protein folding

II. Proprietor of the patent
first named in the patent specification

	University of Chicago
Opponent's or representative's reference (max. 15 keystrokes)	Opposition

III. Opponent

Name	ReMYND NV
Address	Gaston Geenslaan 1 3001 Leuven Belgium
State of residence or of principal place of business	BE
Nationality	BE
Telephone/Fax	+32 16 75 14 16 / +32 16 75 14 21
Multiple opponents (see additional sheet)	☐

IV. Authorisation

1. Representative (name only one representative or name of association of representatives to whom notification is to be made)	
Address of place of business	
Telephone/Fax	
Additional representative(s) on additional sheet/see authorisation	☐

Opponent's reference: Opposition

EPO 2300.1 04.08

2. Name(s) of employee(s) of the opponent authorised to act in these opposition proceedings under Art. 133(3) EPC

Authorisation(s) to 1./2.

not considered necessary ☐

has/have been registered under No. ☐

is/are enclosed ☐

V. Opposition is filed against

• the patent as a whole ☒

• claim(s) No(s).

VI. Grounds for opposition:

Opposition is based on the following grounds:

(a) the subject-matter of the European patent opposed is not patentable (Art. 100(a) EPC) because:

• it is not new (Art. 52(1); Art. 54 EPC) ☒

• it does not involve an inventive step (Art. 52(1); Art. 56 EPC) ☒

• patentability is excluded on other grounds, i.e. Article ☒ Art. 57 EPC

(b) the patent opposed does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Art. 100(b) EPC; see Art. 83 EPC). ☒

(c) the subject-matter of the patent opposed extends beyond the content of the application/of the earlier application as filed (Art. 100(c) EPC, see Art. 123(2) EPC). ☒

VII. Facts (Rule 76(2)(c) EPC) presented in support of the opposition are submitted herewith on a separate sheet (annex 1) ☒

VIII. Other requests:

Oral proceedings are requested if the patent in its entirety is not revoked

Opponent's reference Opposition

IX. Evidence presented

Evidence is enclosed ☒

will be filed at a later date ☐

A. Publications:

1

Particular relevance (page, column, line, fig.):

2

Particular relevance (page, column, line, fig.):

3

Particular relevance (page, column, line, fig.):

4

Particular relevance (page, column, line, fig.):

5

Particular relevance (page, column, line, fig.):

6

Particular relevance (page, column, line, fig.):

Continued on additional sheet ☐

B. Other evidence

Continued on additional sheet ☐

Opponent's reference Opposition

X. Payment of the opposition fee is made

- as indicated in the enclosed voucher for payment of fees and costs (EPO Form 1010) ☒
- via EPO Online Services ☐

XI. List of documents

Enclosure No.

0 Form for notice of opposition ☒

1 Facts (see VII.) ☒

2 Copies of documents presented as evidence (see IX.)

a Publications ☒

b Other documents ☒

3 Signed authorisation(s) (see IV.) ☐

4 Voucher for payment of fees and costs (see X.) ☐

5 Additional sheet(s) ☐ Number of sheets

6 Other ☒

Please specify here:

bank statement - showing the deposit of the opposition fee in the amount of 670 Euro made onto the EPO account DE20 7008 0000 0333 8800 00 on September 23, 2008

XII. Signature of opponent or representative

Place: Leuven

Date: September 30, 2008

Signature

Name (block capitals): DE WITTE, Koen

In case of legal persons, signatory's position within company: Managing Director

Opponent's reference: Opposition

N.V. reMYND
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BTW/VAT BE476.910.101

EPO - Munich
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02. Okt. 2008

European Patent Office
Erhardtstrasse 27
D-80469 Munich
Germany

Fax and TNT delivery

Leuven, 30 september 2008

Our ref.: Opposition

Opposition against European Patent 1 392 849 (02718994.3) - "Yeast screens for agents affecting protein folding" – Applicant: University of Chicago

Dear Madam, dear Sir,

Please find enclosed the "Notice of Opposition" form against European Patent EP 1 392 849 in accordance with Article 99 EPC.

Also enclosed:
- Statement of facts, evidence and arguments – Annex A
- New cited documents (only D9/D10/D11/D13/D14 by fax)
- Form 1010
- Bank statement – showing the deposit of the opposition fee in the amount of 670 Euro made onto the EPO account DE20 7008 0000 0333 8800 00 on September 23, 2008

Yours sincerely,

Koen De Witte
Managing Director

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OPPOSITION to EUROPEAN PATENT No 1 392849
(Application Number 02718994.3)
Patentee: University of Chicago

Dear Sir, Dear Madam,

Notice of opposition under Art. 99 EPC is hereby given by reMYND N.V. to European patent EP-B-0 1 392 849 in the name of University of Chicago. Enclosed is also a filled in EPO form 2.300

Revocation of the patent in its entirety is requested, on the grounds of Art. 100(a) that the subject matter of all claims lack patentability within the terms of Articles 54, 56 and 57 EPC, Art. 100(b) that the patent does not disclose the invention sufficiently clear and complete for it to be carried out by a person skilled in the art and Art. 100(c), that the subject matter extends beyond the content of the application as filed, based on facts, evidence and arguments as set out below.

If the Opposition Division envisages any other decision, oral proceedings are requested.

2. FACTS and EVIDENCE

The opposed patent was filed on 15-02-2002, claiming priority of US provisional application US60/269157 filed on 15-02-01. In the arguments presented below, the following documents will be referred to:

Doc. No.	Publication date	Application date (where applicable)	Publication No./Author/Applicant
D1	19-06-1999	09-12-1998	WO99/29891, Arch Development Corporation)
D2	20-08-1996	15-15-1992	US 5,547,841, Marotta Charles et al.
D3	29-07-1997	05-06-1995	US 5,652 092 Vitek Michael et al.
D4	04-04-1991	12-09-1989	WO91/04339 California Biotechnology

			Inc.
D5	December 1996		Massison DC et al. Trends in Genetics, Elsevier Science Publishers BV, Amsterdam, NL, vol. 12, No. 1, 1996, page 14, XP004037196
D6	01-11-1996		Tuite M et al. Trends in Genetics, Elsevier Science Publishers BV, Amsterdam, NL, vol. 12, No. 11, pages 467-471
D7	5-04-2001	27-09-2000	WO0123412 Massachusetts Inst. Technology
D8	01-02-2001	24-07-2000	WO01/06989 Huston J. et al.
D9	15-08-2000		Osterova-Golts N. et al. J. Neurosc., vol. 20, No. 16:6048-6054
D10	May 1999		Engelender et al., Nature Genetics 22:110-114
D11	15-07-1999		Ostrerova et al. The Journal of Neuroscience 19(14):5782-5791
D12	11-02-1999	31-07-1998	WO99/06545 Max Planck Gesellschaft zur Förderung der Wissenschaften E.V.
D13	15-02-2000		Krobitsch S. and Lindquist S. Cell Biology 97(4):1589-1594
D14	05-08-1999		Liu J.-J. and Lindquist Nature 4000:573-578

Documents D1 to D7 were cited in the International Search Report, and are not enclosed herewith. Documents D8 to D14 are new and are enclosed in duplicate.

3. ARGUMENTS

3.1 The invention and wording of the Claims

The application as filed relates to methods for screening candidate therapeutic compounds for diseases involving amyloid deposition based on cells expressing the

amyloid protein. Two types of screening assays are described, i.e. those wherein the cells are contacted with a toxicity-inducing agent and whereby the effect of candidate compounds on the viability of the cells is detected and those wherein conditions are provided, such as a specific genetic background which ensure aggregation of the amyloid protein and whereby the effect of candidate compounds on aggregation of the protein is detected.

During prosecution of the application, the following independent claims have been submitted, for which a patent has been granted.

Claim 1 of the opposed patent refers to "a method of screening for a compound that decreases alpha synuclein-associated toxicity, the method comprising the following steps:

a) contacting a yeast cell with a candidate compound, wherein the yeast cell expresses a polypeptide comprising alpha synuclein
b) contacting the yeast cell with a toxicity-inducing agent; and
c) evaluating the yeast cell for viability,

wherein viability indicates the candidate compound decreases alpha synuclein-associated toxicity."

With regard to steps a-c, claim 1 corresponds, to the first type of screening assay envisaged, i.e. wherein cells are contacted with a toxicity-inducing agent, generalized to a method for screening for compounds which decrease alpha synuclein-associated toxicity.

Claim 9 of the opposed patent refers to "a method of screening for a compound that decreases alpha synuclein-associated toxicity, the method comprising the following steps:

a) providing a yeast cell engineered to express a polypeptide comprising alpha synuclein
b) contacting the yeast cell with a candidate compound; and
c) evaluating the yeast cell for viability,

wherein an increase in viability of the yeast as compared to viability of the yeast cell in the absence of the candidate compound indicates that the candidate compound decreases alpha synuclein-associated toxicity."

Claim 9 allegedly represents a generalization of the second type of screening assays. However this generalization has led to claims which are now specified to relate to a method for screening for compounds which decrease alpha synuclein-associated toxicity, whereby only the feature of cells engineered to express alpha-synuclein is specified. However, as will be detailed herein this generalization results in a method which a) extends beyond the subject matter of the application as filed and b) in view of its generalization describes a method which can not be carried out successfully by the skilled person. At the same time, as a result of the simplification of the method steps and the open-ended claim language, the claims as granted are found to encompass methods disclosed in and/or suggested by the prior art, resulting in a lack of novelty and inventive step.

3.2 Priority

EP1392849 claims priority of US60/269157. As will be detailed below, Opponent considers that the claims of the granted patent lack basis in the application as filed, and thus are not entitled to the filing date or the priority date claimed. However, to avoid repetition of arguments and in view of the fact that the relevant prior art was published or filed prior to the priority date claimed, arguments contesting the priority claim are not presented at this time. Opponents reserve the right to contest the priority claim at a later stage.

3.3 The claims of the patent lack Novelty contravening Article 100 (a) in combination with Article 52 and Article 54 EPC

3.3.1. Claim 9 lacks Novelty over D8 (WO01/06989)

D8 *(WO01/06989)* was published on February 1st, 2001 and thus is prior art under Article 54(2) for the opposed patent.

D8 discloses screening assays, developed for the identification of intrabodies which bind to amyloid proteins. These assays are described in the section spanning from page 25, line 30 to page 31, line 4, under the heading "IV. screening assays". D8 discloses screening assays for the identification of modulators which bind to a target polypeptide (page 25, line 31-32) and which "alter the undesired accumulation, complexing or aggregation of the selected peptide" (page 25, lines 37-38). Preferred embodiments of the target polypeptide include "alpha-synuclein" (page 26, line 2). In the cell-based assays, the interaction with the polypeptide may be measured as "a change in the biology of the host cell (e.g. a change in levels of cell death)" (page 26, lines 12-15). In the cell-based assays, the use of "a cell which expresses a target polypeptide" is envisaged (page 28, line 21-22). It is stated that "the cell, for example, can be of mammalian origin or a yeast cell" (page 28, lines 24-25).

Accordingly, D8 discloses a method of screening for a compound comprising the steps of:

- *providing a yeast cell engineered to express a polypeptide comprising alpha synuclein*
- *contacting the yeast cell with a candidate compound; and*
- *evaluating the yeast cell for viability*

which corresponds to characterizing steps (a) to (c) of the screening method of claim 9. It is noted in this regard that the remainder of the characterizing portion of claim 9 is redundant as it is clear to the skilled person that a compound which reduces cytotoxicity will increase viability compared to cells without the compound. Accordingly, this feature does not provide any technical contribution to the claim and need not be discussed.
Hence, claim 9 lacks novelty over D8.

3.3.1. Claim 9 lacks Novelty over D7 (WO01/23412)

D7 was filed on September 27th, 2000 and published on April 21st, 2001 and thus constitutes prior art at least under Article 54(3) for the opposed claims.

D7 discloses screening assays, developed for the identification of compounds which disrupt the aggregation of aggregation-disposed polypeptides. Aggregation-disposed polypeptides envisaged in D7 include "synuclein proteins, namely alpha, beta and gamma" (page 11, lines 1-3). The methods disclosed in D7 are described to involve providing a cell that is genetically modified to express a DNA encoding a heterologous polypeptide, contacting the cell with a test compound and determining whether a decrease in the aggregation of the polypeptide occurs in the presence of the test compound (claim 21). Host cells envisaged in D7 include "yeast" cells (page 15, line 8). Methods for detecting if a compound disrupts the polypeptide aggregation include a selection system whereby it is specified that "the ability of the polypeptides to aggregate in the presence of the compound is determined by measuring viability in the presence of a selection agent" (page 21, lines 30-33). It is further specified in this context that an increase in cell viability in the presence of a test compound is an indication that the test compound is an aggregation disrupting polypeptide.

Accordingly, D7 discloses a method of screening for a compound comprising the steps of:

- *providing a yeast cell engineered to express a polypeptide comprising alpha synuclein*
- *contacting the yeast cell with a candidate compound; and*
- *evaluating the yeast cell for viability*

which corresponds to characterizing steps (a) to (c) of the screening method of claim 9. Hence, in view of its broad claim language, claim 9 lacks novelty over D7.

3.4 The claims of the patent lack inventive step contravening Article 100 (a) in combination with Article 56 EPC

3.4.1. Claim 1 lacks inventive step over the disclosure of D8, optionally in combination with D9

Claim 1 of the opposed patent relates to a method for screening a compound that decreases alpha-synuclein mediated toxicity which comprises contacting a yeast cell expressing alpha-synuclein with a candidate compound and contacting the yeast cell

with a toxicity-inducing agent. Dependent claim 2 and 4 specify examples of toxicity inducing agents as including metals and chelators and compounds that cause oxidative stress, respectively.

As detailed above, D8 discloses methods for screening in yeast cells which involve contacting a cell expressing alpha-synuclein as a target polypeptide with a candidate compound and evaluating the effect of the compound on cell viability. The difference between D8 and the method of claim 1 is the step of contacting the cell with a toxicity-inducing agent.
The technical contribution of the toxicity inducing agent is described in the opposed patent as ensuring a toxic phenotype in the yeast cells expressing the amyloid protein, such as alpha-synuclein. D8 generally describes making use of cells expressing amyloid proteins such as alpha-synuclein. D8 does not specify how toxicity of the amyloid protein is ensured.
The problem to be solved, starting from D8 is to provide a screening method wherein toxicity of the expressed alpha-synuclein is ensured, which is of interest in the identification of compounds inhibiting alpha-synuclein-mediated toxicity.

It is noted that D8 describes, also in the section relating to "screening assays", methods which make use of cells or cell cultures from animal models of the disease involving the amyloid protein. D8 suggests the testing of the "sensitivity of these models to compounds such as exocitotoxins or those known to cause oxidative damage" (page 26, lines 33-35. Examples of compounds provided in D8 include metal chelators such as EGTA (Table 1 on page 27). These compounds are described as inducing toxicity. It is submitted that, in view of the context of D8 which relates to the identification of intrabodies capable of binding to or interacting with amyloid proteins, the skilled person will recognize that the cells and cultures of the models envisaged are examples of cells which express the amyloid protein. Accordingly, the skilled person would be motivated to combine this aspect of the disclosure of D8 relating to "tissue slice cultures", with the screening methods on cell cultures (such as can be performed e.g. on yeast cells) disclosed in the preceding paragraphs of D8. This combined teaching, within the same document, discloses a method wherein the effect of a candidate compound on the susceptibility of amyloid protein-expressing cells (e.g. alpha-synuclein-expressing cells)

to a toxic agent is tested. Accordingly, it is submitted that the method of claim 1, if not anticipated, lacks inventive step over the disclosure of D8.

It is moreover submitted that a number of compounds were known at the filing date of the opposed patent to increase the toxicity of alpha-synuclein expression in cells, thus suggesting the use of such compounds in the screening methods of D8. More particularly, D9 (published before the earliest priority date claimed) demonstrates that iron stimulates alpha-synuclein aggregation (D9, Figure 1). Accordingly, it would have been obvious to the skilled person, based on the disclosure of D8 to consider the addition of a toxicity-inducing agent such as disclosed in D9 in the method of D8 so as to be able to determine the effect of the candidate compound on the susceptibility of toxicity-inducing compound, thereby resulting in a screening method having the features of claim 1. Accordingly, it is submitted that claim 1 lacks inventive step over the combination of D8 and D9

3.4.2. Claim 9 and further dependent claims

As will be detailed below, Opponents consider that claim 9 of the opposed patent lacks enablement, in that the skilled person would not be able to carry out the claimed subject matter based on the description provided in the patent application. Indeed, neither the assay as presently presented in granted claim 9, nor the information provided in the description provide the skilled person with sufficient guidance to successfully carry out the claimed method. This is further elaborated under section 3.6 (Sufficiency of Disclosure) herein. It is submitted that the features of the (theoretical) method of claim 9 as presently claimed, correspond more to a desideratum claim and, as such were obvious to the skilled person at the time of filing.

Indeed, as reflected by the background section of the opposed patent, at the filing date it was known that amyloid proteins are involved in the formation of the pathological symptoms of a number of diseases characterized by amyloid deposits and that the identification of compounds which were capable of reducing the toxicity mediated by such proteins would potentially lead to a therapeutic for these diseases. It is detailed herein that it was clear to the skilled person at the time of filing that a screening method

for compounds inhibiting amyloid-protein associated toxicity would comprise at least the features of claim 9.

a) Claim 9 lacks inventive step over the disclosure of D1 (WO99/29891) combined with D10 (Engelender et al.)

The closest prior art for claim 9 is D1. D1 relates to methods of expressing aggregate-prone amyloid proteins in yeast and the use thereof in the screening of inhibitors of aggregation and/or identifying candidate substances with therapeutic activity against amyloidogenic diseases. More particularly D1 describes a screening method based on the induction of the [PSI+] phenotype in yeast by expression of a chimeric protein comprising Sup35 and an amyloidogenic protein.

D1 discloses methods for screening for aggregation inhibitors comprising
- "(a) contacting a yeast cell that expresses an aggregate-prone amyloid protein with said candidate substance under conditions effective to allow aggregated amyloid formation; and
- (b) determining the ability of said candidate substance to inhibit the aggregation of the aggregate-prone amyloid protein." (claim 1 of D1)

In its description D1 specifies that aggregation may be detected in different ways. More particularly it is stated that (page11, lines 12-18):
"*In some preferred embodiments, the [PSI+] phenotype kills the yeast cell. Such cells are particularly useful in screening for the [PSI+] phenotype. For example, yeast expressing a chimeric protein comprising the P-amyloid peptide (1-42) and the Sup35 C-terminal domain have a [PSI+] phenotype that leads to cell death. The inventor contemplates that such cells are an excellent system for screening candidate compounds for their ability to inhibit P-amyloid aggregation, because only yeast grown in the presence of compounds that inhibit or reverse the [PSI+] phenotype will survive.*"
(emphasis added)
Accordingly, D1 discloses methods which comprise "evaluating the yeast cell for viability, wherein an increase in viability of the yeast cell as compared to viability of the yeast cell in the absence of the candidate compound indicates that the candidate compound decreases aggregation."

The difference between D1 and the method of claim 9 is the use of alpha-synuclein as the aggregate-prone amyloid protein in a screening method such as the one disclosed in D1. The problem to be solved corresponding to this difference is the recognition of alpha-synuclein as a suitable aggregate-prone amyloid protein.

There are different documents which indicate that alpha-synuclein was known as an aggregate-prone amyloid protein, prior to 2001.

The disclosure of Engelender et al. (D10) demonstrates that in eukaryotic cells, co-expression with synphilin-2 promotes aggregation, very similar to what is described for the chimeric proteins comprising amyloid proteins in D1. Accordingly, based on the disclosure of D10 the skilled person would recognize alpha-synuclein as an aggregate-prone protein, and would be motivated to develop a screening method for agents inhibiting aggregation of alpha-synuclein in yeast using the screening method of D1. Moreover, based on the disclosure of D9, which generally lists a number of amyloidogenic proteins and the disclosure of D10 demonstrating the aggregation of alpha-synuclein in eukaryotic cells, the skilled person would have a reasonable expectation of success when applying the methods of D1 with alpha-synuclein as the aggregate-prone protein.

Accordingly, it is considered that applying the methods of D1 making use of an alternative aggregate-prone protein such as alpha-synuclein known inter alia from D10 in a screening method to identify inhibitors of toxicity of alpha-synuclein does not involve an inventive step.

b) Claims 9-11 lack inventive step over the disclosure of D1 (WO99/29891) combined with D11 (Ostrerova et al.).

The disclosure of D1 and the problem to be solved starting from D1 is detailed above. D11 discloses the toxicity of overexpression of alpha-synuclein in 293 HEK cells (abstract, right column, lines 4-5). D11 further discloses the toxicity of the A53T and A30P mutants. Accordingly, it is submitted that based on the disclosure of D11, the skilled person would recognize alpha-synuclein as an aggregate-protein protein, and

would consider performing the method of D1 using alpha-synuclein (or one of the known mutants thereof), thus obtaining a method falling within the broad language of claim 9. Accordingly, claims 9-11 lack inventive step.

c) Claim 9 lacks inventive step over the disclosure of D12 (WO99/06545) in view of D8 (WO01/06989)

D12 was published on February 11th, 1999 and thus constitutes prior art under Article 54(2) for the opposed claims.

D12 relates to aggregate formation by amyloidogenic proteins and discloses methods for testing a prospective inhibitor of aggregate formation of a non-cleaved fusion amyloidogenic polypeptide which involve incubating, in the presence of a prospective inhibitor, said fusion poly(peptide) and assessing the formation of amyloid-like fibrils or protein aggregates (D12, page 15, lines 20-28 and claim 16). D12 envisages methods which involve making use of a composition which is a host transformed with a vector containing a nucleic acid molecule encoding fusion protein encoding an amyloidogenic polypeptide that has the ability to self- assemble into amyloid-like fibrils or protein aggregates (claim 1, D12). Examples of amyloidogenic (poly)peptides envisaged in D12 include α-synuclein (claim 7, D12) and yeast cells are envisaged as host cells (claim 11, D12).
It is noted that, in the background section, D12 indicates that the mechanism underlying Huntington's Disease (HD) are likely to be similar to those in Alzheimer's Disease, "in which "beta"-sheet secondary structures lead to the formation of toxic protein aggregates in selective neurons" (page 3, last sentence of first paragraph of D12).

Accordingly, D12 discloses a method of screening for an inhibitor of aggregate formation comprising the steps of

- *providing a yeast cell engineered to express a polypeptide comprising alpha synuclein, and*
- *contacting the yeast cell with a candidate compound*

The difference between D12 and claim 9 is that in D12, the methods envisaged involve "assessing the formation of amyloid-like fibrils or protein aggregates" rather than determining viability of the cells. The technical contribution of screening for viability is

indicated in the opposed patent as the fact that "an understanding of the physiology and/or cell biology of the misfolded disease protein or the etiology of a misfolded protein disease is not necessary to identify candidate therapeutic compounds"

The problem to be solved starting from D12 is thus to provide a screening method which allows straightforward identification of the effect of the compounds, which does not require assessing aggregation.

This problem is solved in D8. Indeed, as indicated above, D8 in fact generally discloses screening methods encompassed by claim 9. Moreover however, D8 particularly specifies that "the ability of the polypeptides to aggregate in the presence of the compound is determined by measuring viability in the presence of a selection agent" (page 21, lines 30-33). It is further specified in this context that "an increase in cell viability in the presence of a test compound plus selection agent, compared to the selection agent alone, is an indication that the test compound is an aggregation disrupting polypeptide".

Accordingly, D8 discloses methods whereby aggregation can be detected based on cell viability.

In view of the above, it is considered that the methods of claim 9 are obvious over the combination of D12 and D8.

d) The problem is not solved over the entire scope of the claim

The underlying technical problem according to the application as filed was presented to develop screening methods in yeast which make it possible to screen compounds for their therapeutic potential in AD based on their ability to increase viability in cells which display alpha-synuclein mediated toxicity. Screening methods were developed whereby 'agents/conditions' induce toxicity of amyloid proteins such as alpha-synuclein.

Claim 9 as granted however relates to screening methods on yeast cells which methods are essentially only characterized by the use of cells which express alpha-synuclein. No toxicity-inducing agents or conditions are specified. Accordingly, the claim now

encompasses methods wherein alpha synuclein is expressed e.g. in the absence of specific toxicity inducing agents or conditions.

Methods wherein cells are used which do not display alpha-synuclein mediated toxicity, will not be useful in the identification of compounds capable of reducing alpha-synuclein mediated toxicity. In fact, as claim 9 fails to specify which conditions should be implemented so as to induce alpha-synuclein associated toxicity, it encompasses methods whereby other types of toxic agents are added to the cells. In such methods however, a compound resulting in an increase in viability compared to controls can at most be considered to be a compound capable of neutralizing the effect of the toxic agent. Accordingly such methods do not allow the identification of compounds with therapeutic potential or capable of decreasing alpha synuclein associated toxicity.

Accordingly, opponents consider that the problem is not solved over the entire scope of claim 9.

3.4.3. Claims 1-11 lack inventive step in view of any one of D1, D8, D11, 12, D13, D14 or combinations thereof

For the same reasons as disclosed above the combinations of the prior art documents described above makes the claims of the patent in suit obvious to a person skilled in the art.

D13 (Krobitsch and Lundquist, 2000) and D14 (Liu and Lindquist, 1999) are publications dating before the earliest priority date claimed in the opposed patent, which emphasize the utility of yeast as a valuable system for investigating factors that affect aggregation and for determining factors which might influence toxicity (D13, page 1589, right column, last sentence of introduction) as well as the emphasizing the similarities of the aggregation process between mammals and yeast (D14, page 575, left column, last complete sentence). Accordingly, it is submitted that, would the Opposition Division have any doubts as to whether the skilled person would be motivated to try or expect similar results performing the prior art methods in yeast (for which there is however no indication in the prior art documents), this is addressed by these documents which were known to the skilled person at the date of filing.

3.5 The claims of the patent lack Industrial Applicability contravening Article 100 (a) in combination with Article 57 EPC

Claim 1 defines a method which cannot be considered susceptible of industrial application because a result to be achieved is being claimed.

Indeed, claim 9 relates to a method for screening a compound that decreases alpha-synuclein-associated toxicity. The screening is based on observing increased viability. It is clear that increased viability is a measure for determining the ability of a compound to decrease toxicity. However, the characterizing features of the method itself are not disclosed and the result is merely defined by the object of the process. It is submitted that claim 9 is an example of circular reasoning, and therefore cannot be susceptible of industrial application.

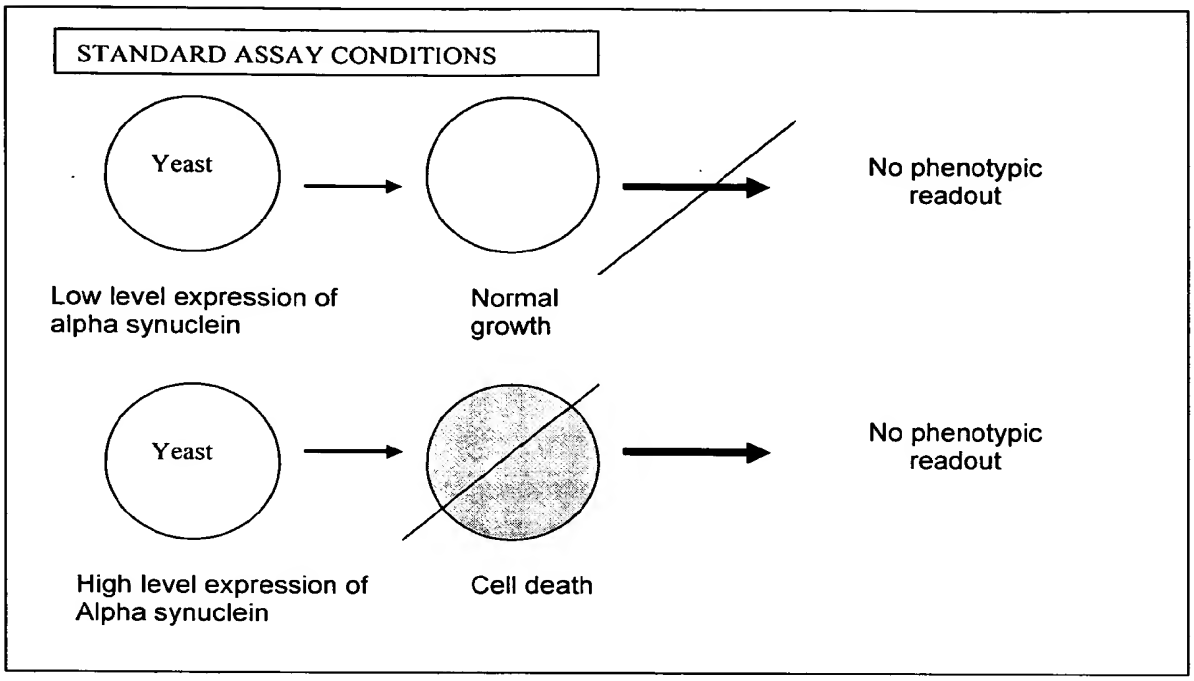
3.6 The patent does not disclose the invention sufficiently clear and complete for it to be carried out by a person skilled in the art, contravening Art. 100(b) EPC.

Opponents consider that there can not be considered to be sufficient guidance in the application as filed to carry out the invention as presently claimed in claim 9.

Claim 9 as granted recites only the following essential features: providing yeast cells engineered to express a polypeptide comprising alpha synuclein, contacting these cells with a candidate compound and assessing the viability of the cells.

It is noted that, while low levels expression of alpha-synuclein will not lead to aggregation, expression of alpha-synuclein at a level resulting in aggregate formation is toxic for the cell (see, inter alia D9). Accordingly, upon performing step (a) of the method of claim 9, i.e. expressing alpha synuclein in yeast, one will counter-select the yeast cells for low expression levels of alpha synuclein, which does not aggregate and as such is

not toxic to the cells. As a result, it is not possible to evaluate the effect of a compound on viability. This is illustrated schematically below.



The application as filed of the opposed patent describes methods wherein additional features ensure the toxicity of alpha synuclein, such as co-expression with hsp40, the expression of mutant forms of alpha synuclein and/or fusion proteins under control of an inducible promoter, or the addition of a toxicity inducing agent. Indeed, the examples of EP 1 392 849 relating to screening methods envisage either the use of a toxicity inducing agent (Example 11) or expression as a fusion protein, under control of an inducible promoter (Example 12). It appears however that the presence of one of these additional features is critical to ensuring that the yeast cell line demonstrates alpha-synuclein-associated toxicity such that compounds can be screened for their ability to reduce alpha synuclein-associated toxicity.

Neither the application, nor any post-published documents provide any guidance to the skilled person for performing methods characterised by only the steps as specified in claim 9, i.e. expressing alpha synuclein and determining an increase in viability. Thus

there are serious doubts as to whether the method can be carried out over the whole range claimed (T612/92, T694/92).

3.7. The subject matter of the claims extends beyond the subject matter of the application as filed contravening Article 100(c).

3.7.1. Lack of disclosure for methods of screening for a compound that 'decreases alpha-synuclein toxicity" (claims 1 and 9)

Independent claims 1 and 9 as granted both relate to methods of screening, whereby the method is identified as a method of screening for a compound that decreases "alpha-synuclein toxicity". The result of the screening method is the identification of an agent as decreasing alpha-synuclein-associated toxicity.

First it is noted that the application as filed does not disclose methods for identifying an agent as decreasing alpha-synuclein-associated activity. Indeed all of the screening methods disclosed relate to methods for identifying therapeutic agents. The outcome of these assays is the identification of a candidate therapeutic agent. It is clear that with the use of the general wording relating to 'alpha-synuclein-associated toxicity" the patentee aims to expand the scope of the claims beyond that of the application as filed.

Moreover, the application as filed generally describes methods of screening for a therapeutic agent for "protein misfolding diseases". These methods comprise, *inter alia*, the use of a yeast cell expressing a polypeptide comprising a "misfolded disease protein". Several examples of protein misfolding diseases and misfolded disease proteins are generally disclosed. However, throughout the description methods which make use of yeast cells comprising alpha-synuclein are described as methods for identifying therapeutic agents for the treatment of Parkinson's disease.

Similarly, Claims 30 and 53 of the application as filed which relate to screening methods involving yeast cells comprising alpha-synuclein, are described as methods "for identifying therapeutic agents for the treatment of Parkinson's disease". Again, while the original application only linked alpha-synuclein toxicity to the treatment of Parkinson's

disease the patentee has extended the scope of the claims to encompass screening methods for any type of disease, and to encompass screening methods for identifying research tools. At the same time this implicitly affects the type of compounds which can be screened, i.e. the screened compounds are no longer limited to candidate therapeutic agents) as envisaged in the application as filed.

Accordingly, the subject matter of all of the granted claims extends beyond the subject matter of the application as filed, violating Article 123(2).

3.7.2. Lack of Support for the method steps of claim 9

Claim 9 of EP1392849B1, was filed by the Applicant (now patentee) during the prosecution of the application, on March 9, 2007, as a *new* claim (i.e. no claim previously on file was provided as a basis). Claim 9 contains the following features:

A method of
- screening for a compound that decreases alpha synuclein-associated toxicity,

the method comprising the following steps:
- a) providing a yeast cell engineered to express a polypeptide comprising alpha synuclein;
- b) contacting the yeast cell with a candidate compound; and
- wherein an increase in viability of the yeast cell as compared to viability of the yeast cell in the absence of the candidate compound indicates that the candidate compound decreases alpha synuclein-associated toxicity.

In the accompanying letter filed by the applicant at the EPO it was stated that the disclosure of new claim 9 was to be found on page 11, lines 15 – 21 of the WO specification (i.e. WO02065136).

This passage is recited below:

The present inventors have developed a system which allows the rapid identification of candidate therapeutic agents that prevent and/or inhibit the process of protein aggregation leading to fibrillogenesis and protein deposition. The system is a yeast-based system, wherein a yeast cell is engineered to expresses a protein or polypeptide that is involved in fibril formation, for example, the yeast cell can express a huntingtin polypeptide in the case of Huntington's disorder, or expresses an alpha synuclein polypeptide in the case of Parkinson's disease, or express an amyloid protein in the case of a disease involving amyloidoses (also see Table 1 for a list of proteins that are associated with fibril formation). In addition to this, in one

It is noted that the passage indicated by the applicant (i.e. page 11, lines 15 – 21 of the WO specification) does not disclose a method having all of the features of new claim 9. To the contrary, this passage of the specification merely generally refers to the screening methods which have been developed and does not recite any method steps. Accordingly, this passage can not be considered to provide support for the wording of claim 9. Moreover, it is clear from the above excerpt of the description that the description goes on to recite further essential features of the methods of the invention, which are not included in the citation. Indeed, picking up on the last line of the paragraph recited above, this section continues as follows up to page 13, line 10 (emphasis added):

Table 1 for a list of proteins that are associated with fibril formation). <u>In addition to this</u>, in one embodiment, the yeast cell also has a genetic background that causes the yeast cell to have reduced growth rates or no growth as a result of expressing the recombinant polypeptide in combination with the genetic background. In one example, the yeast cell has a mutant Hsp40

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In an alternative embodiment, the yeast cell expressing the recombinant fibril forming protein or polypeptide, is exposed to a set of growth conditions that causes the yeast cell to have reduced or no growth. For example, one may contact the yeast cell with iron or a free radical generator that causes oxidative stress to the cell. Again, a candidate substance can be contacted with this yeast cell to screen for potential agents that can reverse yeast cytotoxicity, which is also correlated to the ability of the agent to prevent cytotoxic protein aggregation and fibril formation.

By referring to a particular section on page 11 of the specification, the Applicant (now Patentee) has attempted to isolate the section from what follows, wherein the actual

embodiments are described. This isolated section does not provide support for the method steps recited and does not reflect the actual disclosure of the application as filed. Indeed, in other sections of the description, the requirement of further features is emphasized, e.g. the section spanning page 48, line 27 to page 49, line 2 (emphasis added):

> The screening methods of the invention use yeast cells that are engineered to express proteins involved in fibril formation and/or in protein aggregation. The yeast cell also requires one of the two conditions described below for the screening method. In one module, the yeast cell have a mutant genetic background, for example, mutations in HSP genes or other molecular chaperone encoding genes, such as mutations in the HSP40 gene. Alternatively, the yeast cell expressing a protein involved in fibril formation and/or in protein aggregation can be subject to changes in growth conditions that lead to stress, such as oxidative stress, for example by exposing the cell to a free radical generator, or iron etc. Either of these conditions confers a toxic phenotype on the yeast cells expressing proteins involved in fibril formation and/or in protein aggregation.

This passage of the description clearly reflects that the application as filed envisaged that particular conditions were required to ensure a toxic phenotype to the cell.

In addition to these passages of the description, the disclosure of the application as filed is moreover reflected by the claims which were filed together with the application. Of these, only claims 30, 53 and 54 refer to a method of screening which involve yeast cells comprising alpha-synuclein. These methods are specified as methods for screening compounds to identify a therapeutic agent for Parkinson's disease or for a protein misfolding disease (which can be Parkinson's disease according to a subclaim), respectively.

Claims 30 and 54 comprise, as mandatory features, the step of:

(i) contacting the yeast cell with a toxicity inducing agent.

In contrast thereto, claim 53 comprises, as mandatory features, the steps of:

(ii) incubating the yeast cell under conditions that allow for aggregation of the polypeptide;

(iii) measuring the aggregation of the polypeptide; and

(iv) comparing the level of aggregation with the level of aggregation in a yeast cell not contacted with the candidate compound.

These claims reflect the disclosure of the application as filed, i.e. the methods envisaged at the time of filing were to involve either the use of a toxicity-inducing agent or conditions which allow for aggregation of the polypeptide (whereby aggregation rather than toxicity is measured). More particularly, methods of screening involving alpha-synuclein are only disclosed in the application as filed in combination with either step (i) or steps (ii) – (iv), as set forth above. Accordingly, removal of these steps in the method of claim 9 was contrary to Article 123(2) EPC.

4. CONCLUSION

For the reasons set out above, revocation of European Patent No. 1 392 849 in its entirety is requested. If the Opposition Division envisages any other decision, oral proceedings are requested.